



Butler, M., Down, C. J., Foster, R. R., & Satchell, S. C. (2020). The pathological relevance of increased endothelial glycocalyx permeability. *American Journal of Pathology*, 190(4), 742-751. <https://doi.org/10.1016/j.ajpath.2019.11.015>

Peer reviewed version

License (if available):
CC BY-NC-ND

Link to published version (if available):
[10.1016/j.ajpath.2019.11.015](https://doi.org/10.1016/j.ajpath.2019.11.015)

[Link to publication record in Explore Bristol Research](#)
PDF-document

This is the author accepted manuscript (AAM). The final published version (version of record) is available online via Elsevier at <https://doi.org/10.1016/j.ajpath.2019.11.015> . Please refer to any applicable terms of use of the publisher.

University of Bristol - Explore Bristol Research

General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available: <http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

Title Page

The pathological relevance of increased endothelial glycocalyx permeability

Running head Glycocalyx regulates permeability

Matthew J Butler^{1*A}, Colin J Down*, Rebecca R Foster*, Simon C Satchell*

* Bristol Renal, Translational Health Sciences, Bristol Medical School, University of Bristol, UK

¹ Co-corresponding author (matthew.butler@bristol.ac.uk)

Funding

^A NIHR funded clinical lecturer in renal medicine

Details

Text page numbers = 11, 22 (including references)

Number of figures = 3

Abstract

The endothelial glycocalyx is a vital regulator of vascular protein permeability and hydraulic conductivity. Damage to this delicate layer can result in increased protein and water transit. The clinical importance of albuminuria as a predictor of kidney disease progression and vascular disease has driven research in this area. In this review we outline how research to date has attempted to measure the contribution of the endothelial glycocalyx to vessel wall permeability. We discuss the contrasting published results, comparing evidence for the role of the endothelial glycocalyx in regulating permeability in discrete areas of the vasculature and highlight the inherent limitations of the data that has been produced to date. In particular we draw the readers attention to the difficulties in interpreting a normal urinary albumin level in early disease models. In addition, we summarise the research supporting the view that glycocalyx damage is a key pathological step in a diverse array of clinical conditions including diabetic complications, sepsis, preeclampsia and atherosclerosis. Finally, we discuss novel methodologies including an *ex vivo* glomerular permeability assay that should advance our understanding of permeability changes in disease.

Introduction

Water and solute exchange across the walls of the microcirculation are dynamic processes that are fundamental to tissue homeostasis. The net rates of exchange are regulated by alterations in systemic blood pressure (hydrostatic pressure), vessel density and size (vessel surface area), flow rate and concentration gradients, and the inherent permeability of the vessel walls. The basic structure of the capillary wall is conserved throughout the body, consisting of an endothelial cell monolayer, basement membrane and supporting cells. However, a high level of specialisation occurs within discrete areas of the vasculature, optimising the structure for the individual demands placed upon it. The endothelial glycocalyx layer on the luminal surface of the endothelial cells contributes to the permeability barrier formed by the vessel wall.^{1,2} Glycocalyx literally translates from the Greek for 'sugar coat' glykys = sweet, kalyx = husk. This adherent structure includes proteoglycans, glycoproteins and glycolipids (figure 1).^{1,3-5} Proteoglycans consist of core proteins (e.g. syndecans and glypicans) with covalently-bound glycosaminoglycan side chains (e.g. heparan sulphate (HS) and chondroitin sulphate). The glycocalyx is not uniform across its depth.⁶ The two-layer fibre-matrix model suggests a dense 200-300nm mesh-like inner layer, rich in proteoglycans covalently bound to the endothelial cell membrane and adherent glycosaminoglycans including long chains of hyaluronan (HA), and an outer more porous gel-like layer up to 1µm thick including adsorbed plasma proteins.^{6,7} Here we review the evidence for the importance of the endothelial glycocalyx as a regulator of endothelial and vascular permeability whilst noting the inherent difficulties in studying it. We then discuss the human diseases where glycocalyx damage and associated permeability alterations appear to be key pathogenic steps.

The complexities of studying the endothelial glycocalyx as a permeability barrier

Endothelial cells *in vitro* produce a surface glycocalyx providing an accessible model to study glycocalyx functions including shear stress sensing² and permeability regulation.⁴ However it is much thinner than the glycocalyx seen *in vivo* limiting the applicability of *in vitro* research.^{4,8,9} As a result the majority of work studying the permeability of the glycocalyx has been conducted *in vivo*.

Much research to date has focussed on the glomerular endothelial glycocalyx. This may be driven by our appreciation of the clinical importance of albuminuria and the ease with which urinary albumin creatinine ratios (uACR) can be quantified to provide a measure of albumin permeability across the glomerular filtration barrier (GFB) (figure 1). Early models of the GFB overestimated the contribution of the slit diaphragm to the restriction of albumin filtration.¹⁰ Recent models of the GFB, however, suggest that the glycocalyx represents a significant

protein barrier.¹¹ This concurs with accumulating experimental evidence,¹² indicating that the remaining components of the GFB, including the endothelial glycocalyx, provide the major barrier to protein permeability (figure 1).^{4,13-15} These estimates, combined with our increased understanding of cellular crosstalk within the GFB should make us question assumptions about the pathogenesis of albuminuria in multiple historical models.^{16,17} Albuminuria is the net result of albumin passage across the GFB (influenced by permeability), and albumin reabsorption and metabolism within the renal tubules. However, despite the widespread use of uACR, there is increasing evidence in rodent models that the uACR is not a sensitive test for changes in GFB permeability and hence not an ideal index of glycocalyx integrity: Using *in vivo* multiphoton microscopy in mice (figure 2) we have demonstrated that glomerular albumin leakage can be significantly increased before detectable levels of albumin appear in the urine.² Other groups have reported similar data, demonstrating increased glomerular albumin leakage but no significant change in uACR.¹⁸ We hypothesise that this discrepancy is explained by tubular re-absorption of filtered albumin, resulting in a threshold effect whereby increased glomerular albumin permeability will not result in uACR increases until mechanisms of uptake and metabolism are overwhelmed. The role of tubular albumin uptake in humans is debated, but diseases resulting in tubular dysfunction, such as Fanconi syndrome and Dent's disease do result in significant albuminuria.¹⁹ Historical studies in diabetic patients using lysine to inhibit tubular albumin re-absorption similarly indicate that tubular albumin uptake may influence uACR results.²⁰ In summary the clinical importance of albuminuria is now well established, but the absence of albuminuria in early rodent models of disease does not exclude increased albumin passage across the GFB.

Relatively little research has been conducted studying glycocalyx-dependant permeability changes in the systemic vasculature. In part this is due to the complex methods needed to study glycocalyx specific changes. We have previously studied mesenteric micro-vessels in real time using confocal microscopy, but due to the fragility of the glycocalyx layer careful tissue preparation is needed.¹ Work comparing the depth of the endothelial glycocalyx within the continuous capillaries of the systemic and pulmonary vasculature highlighted dramatic variability in glycocalyx depth.²¹ To date little is known about whether the composition of the glycocalyx varies between these sites. It seems likely that the glycocalyx within discrete areas of our vasculature is specialised, adapting to perform the combination of tasks needed at each tissue site optimally. Variability in the glycocalyx structure means that pathological insults may not affect all areas of the glycocalyx equally. Specialisation is also seen in the endothelial monolayer itself. The 'double barrier' concept was first introduced by Rehm et al and is illustrated in figure 3.²² They found in guinea pig hearts that simultaneous disruption of both the cellular barrier (using ischaemia or histamine) and glycocalyx damage (using heparinase)

was needed to increase coronary vessel leakage.²² This work led to the hypothesis that glycocalyx damage overlying a tight cellular barrier will have minimal (direct) influence on monolayer or vessel wall permeability. In contrast, identical glycocalyx damage overlying a 'leaky' cellular monolayer will result in rapid measurable increases in vessel permeability. When studying glycocalyx-dependant permeability changes within the systemic vasculature it is therefore important to consider both how glycocalyx structural adaptations and the underlying endothelial cell phenotype will influence detectable permeability changes.

To measure the contribution of the endothelial glycocalyx to vessel permeability comparisons have generally been made following a 'glycocalyx insult'. Enzymatic removal or genetic knock down of a specific glycocalyx component is commonly used for this purpose. However, the method used can dramatically alter the results of such studies. Rapid removal of HS, using human heparanase or heparanase III, increased albumin passage across endothelial monolayers.⁴ However knock out of the HS proteoglycan syndecan 1 did not result in albuminuria in mice.²³ In addition, mice lacking endothelial N-Deacetylase and N-Sulfotransferase (NDST-1), a key enzyme in modifying HS chains, did not become albuminuric.²⁴ A similar pattern of results was seen in experiments targeting HA. Hyaluronidase has been shown to have a plasma half-life of approximately 3 minutes before being taken up in the liver via a mannose-dependant mechanism.²⁵ The short plasma half-life and the relatively high molecular weight (55-61kDa) of hyaluronidase result in a high level of glycocalyx-degrading activity with limited 'off target' effects. Haraldsson et al found that removing HA from the endothelial glycocalyx using hyaluronidase increased glomerular albumin transit 5.6-fold, a figure consistent with our own findings.²⁶⁻²⁸ Tamoxifen induced endothelial specific knock down of hyaluronan synthase 2 (HAS2) also resulted in significant albuminuria from 4 weeks post induction, which persisted to 12 weeks (experimental end point). In contrast, Dane et al showed that 4 weeks hyaluronidase infusion did not result in measurable albuminuria although they did demonstrate increased glomerular albumin leakage in 90% of glomeruli.¹⁸ Whilst this work again highlights that albuminuria is a poor measure of low-level glomerular albumin leakage, it also suggests that the differences in time-scale, as well as the precise component targeted may influence glycocalyx permeability changes. Knockdown in genetic models may have resulted in compensatory adaptations e.g. up-regulation of other glycocalyx components. In contrast the rapid enzymatic removal of glycocalyx components may prevent adaptations from occurring before functional assessments are made.^{24,26-}

²⁹ In addition, the rapid removal of a single structural component of the glycocalyx may leave the structure as a whole vulnerable to further non-specific destruction by the shear forces applied by the circulation. Another contrast between enzymatic removal and genetic knock down of a glycocalyx component is the generation of circulating glycocalyx fragments. The enzymatic release of fragments is also non-specific. Heparanase

increased syndecan 1 and 4 loss from the glycocalyx structure, possibly by exposing cleavage sites for the actions of other circulating enzymes.³⁰ The influence of active signalling fragments released following glycocalyx enzymatic degradation on vessel permeability has not been directly investigated, but they represent a potentially important pharmacological target.^{31,32}

In summary, when studying the glycocalyx careful consideration needs to be given to the chosen methodology and tissue. Whilst the glomerular endothelial glycocalyx lends itself to studying permeability the uACR should be interpreted with caution. Measures of systemic permeability need to be considered in context. A 'double barrier' can exist and in the absence of specific manipulations, the relative contributions of the components are difficult to establish. With the development of more sensitive and specific methods to assess permeability we expect our understanding of the role of individual glycocalyx components to rapidly increase in the next decade.³³

The relevance of glycocalyx permeability changes in disease

Diabetes

There is evidence that the diabetic milieu affects the vasculature globally. Nieuwdorp et al measured the total glycocalyx volume by comparing the volume of distribution of erythrocytes and dextran 40 in healthy volunteers. They found that 6 hours of hyperglycaemia reduced the glycocalyx volume to 50% of the baseline value.³⁴ More recently an intervention study has shown that improved glycaemic control in type 2 diabetic patients (for 12 months) results in a significant increase in glycocalyx depth. Glycocalyx depth in this study was measured using side-stream dark field imaging (SDF) to assess the perfused boundary region depth (a measure inversely proportional to glycocalyx thickness) on sublingual vessels. This suggests that systemic glycocalyx damage in early diabetes is at least partially reversible.³⁵ We have found that changes to the endothelial glycocalyx occur early in the disease course, suggesting that they could represent a valuable direct therapeutic target.³³ Using electron microscopy on glomerular capillaries we have shown that the percentage surface covered by the glycocalyx is one of the earliest detectable vascular changes in diabetes.³³ Consistent with this Targosz-Korecka et al used atomic force microscopy to 'map' the glycocalyx on diabetic db/db mouse aortas.³⁶ They found that endothelial glycocalyx coverage was significantly reduced by week 11.³⁶ At this time point significant depth reductions in the glycocalyx structure were not detected.³⁶ Although glycocalyx damage occurs early in diabetic disease, the clinical manifestations of this damage may be significantly delayed and remain dependent on the function of the vascular bed studied.

Diabetic nephropathy (DN)

The hallmark of DN is micro-albuminuria. In diabetes the uACR is a key screening test, predicting patients at the highest risk of progressive renal and vascular disease.³⁷ Using electron microscopy to study glomerular structural changes in diabetic patients, loss of endothelial fenestration area was found to correlate with the uACR more strongly than podocyte detachment.³⁸ These data suggest that endothelial damage is likely to be one of the key steps that results in albuminuria in diabetes.³⁸ The authors of this study did not examine the glycocalyx, but it seems likely that the endothelial cell damage, resulting in fenestration loss, was the result of altered vascular endothelial growth factor (VEGF) signalling.^{39,40} We have shown that altered VEGF signalling results in glycocalyx loss.^{15,28,41} Given the evidence supporting the importance of the glycocalyx to glomerular filtration, it seems likely that glycocalyx damage contributed to the micro-albuminuria seen in these patients.

Experimental evidence supports this view. On cultured monolayers of conditionally immortalised glomerular endothelial cells (GEnC) we have shown that high concentrations of glucose result in a marked reduction in the biosynthesis of the glycocalyx component HS with an associated increase in albumin permeability.⁴² Jeansson et al confirmed that increased glomerular albumin leakage contributed to the albuminuria detected in animal models of diabetes by cooling isolated kidneys to 8°C to limit tubular effects.⁴³ Loss of HA and HS from the glomerular capillary wall has been confirmed in streptozocin (STZ) induced diabetic rats and Zucker fatty rats suggesting that glycocalyx damage is likely to contribute to the increase in albumin filtration seen in these disease models.^{44,45} Using high power electron microscopy we have shown in rodent diabetic models that glomerular endothelial glycocalyx changes occur before any alterations to the other components of the filtration barrier.^{28,33} At this early time point, using an isolated glomerular albumin permeability assay, we have confirmed that glomerular glycocalyx loss is associated with increased glomerular albumin permeability in the STZ diabetic model.²⁸ This unique assay has the advantage of studying glomerular permeability in the absence of haemodynamic influence, ensuring that alterations in the glomerular perfusion pressure will not influence permeability measurements. In addition, this assay allows us to study changes in glomerular permeability in isolation from the renal tubules. As discussed, we feel this is important in early disease models with low-level albuminuria. Using this assay, we found that glomerular albumin leakage may be significantly increased before a rise in uACR is detectable. In summary, the current evidence suggests that glycocalyx injury is likely to be contributory to the early pathogenesis of DN.

Diabetic retinopathy (DR)

Commented [SS1]: Reference Raina's recently accepted paper in this section

Add when available

Diabetic retinopathy, like DN, is a manifestation of diabetic microvascular damage. The retina is a continuation of the central nervous system with a blood retina barrier. The pathogenesis of DR has been reviewed in detail elsewhere,^{46,47} but the role of the retinal endothelial glycocalyx, and its impairment in diabetes, is an area of on going research. DR is characterised by micro-aneurysms, leukocyte-endothelial adhesion, haemorrhage, capillary occlusion, neovascularisation and increased permeability.⁴⁸ Diabetic rats have a significantly thinner retinal endothelial glycocalyx compared to control rats (28.3nM vs. 60.2nM $p<0.01$), measured using transmission electron microscopy.⁴⁹ In addition, in the Akita mouse model of type 1 diabetes, anaesthetised diabetic mice have a significantly thinner glycocalyx in retinal arterioles.⁴⁸ In human volunteers, combined fluorescein/indocyanine green angiography also demonstrated a significantly thinner glycocalyx in patients with type 2 diabetes compared to healthy controls.⁵⁰ In the same study the authors measured the rate at which ¹²⁵I-labelled albumin left the plasma, confirming an increased rate of loss in diabetic patients – consistent with an increase in systemic albumin permeability.⁵⁰ Although a reduction in tight junctions within the inter-endothelial cleft of retinal vessels has also been reported early in diabetic disease, it seems likely that glycocalyx damage contributes to microvascular permeability changes in DR.⁵¹

Sepsis

During sepsis the endothelial glycocalyx becomes thinner and the cover more sparse, contributing to tissue oedema.^{52,53} In mice, intravenous lipopolysaccharide (LPS) injection results in activation of the innate immune system, shock, lactic acidosis, myocardial impairment and increased levels of circulating tumour necrosis factor alpha (TNF α) and interleukin-6 (IL-6).⁵⁴ Following LPS administration mice have significantly thinner aortic glycocalyx compared to controls.⁵⁵ Elevated levels of TNF α are likely to contribute to glycocalyx damage in this model via increased matrix metalloprotease activity and syndecan loss.⁵⁶ In human volunteers a low-dose endotoxin model resulted in a significant reduction in the depth of the sublingual vessel glycocalyx (measured using side stream imaging) and a concurrent elevation in plasma hyaluronan, suggesting glycocalyx shedding.⁵⁷ Multiple biomarkers of glycocalyx shedding have been studied in humans as markers of sepsis including syndecan 1, HS and HA.^{52,58,59} It is hoped that in the future these targets may provide clinicians with additional diagnostic and prognostic information. In sepsis circulating sheddases including A disintegrin and metalloproteinase 15 (ADAM15), heparanase, and matrix metalloprotease (MMP) 2 and 9 have been implicated in the degradation of the glycocalyx.⁵⁹⁻⁶² It seems likely therefore that the albuminuria observed in sepsis results from glycocalyx injury and hence increased glomerular albumin permeability.^{14,63-65}

Increased pulmonary vascular permeability in sepsis manifests as acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), both can occur early in sepsis.⁶⁶ However, the evidence to date suggests that we should not extrapolate evidence supporting peripheral glycocalyx damage mediating increased vasculature permeability to the pulmonary vasculature in sepsis. Mouse pulmonary endothelial glycocalyx appears to be substantially thicker than that seen in the mouse systemic vessels (cremaster muscle).⁶⁷ Conflicting evidence exists supporting the role of the pulmonary vessel glycocalyx as a permeability barrier. Whilst a study using bovine lung microvasculature endothelial cells *in vitro* suggested that HS within the glycocalyx contributed to cell's barrier function, a study using *ex vivo* rat lungs did not.^{68,69} In addition, in isolated mouse lungs perfused with 4% Evans blue labelled albumin, neither water nor albumin permeability was measurably altered by glycocalyx degradation. *In vivo* heparinase-III enzyme infusion did not result in detectable pulmonary oedema in mice.⁷⁰ These findings could be explained by the double barrier hypothesis (figure 3). Glycocalyx injury in isolation may not result in measurable increases in pulmonary permeability to macromolecules in the presence of an intact second barrier (the endothelial monolayer). However, by altering the level of immune cell extravasation and inflammation, the pulmonary glycocalyx may still have a key role in maintaining pulmonary homeostasis.⁷⁰

The endothelial cells within the cerebral circulation form part of the blood brain barrier, limiting transcytosis and solute diffusion.⁷¹ The glycocalyx forms the most luminal layer of the blood brain barrier. Whilst cytokines smaller than 40kDa are likely to freely cross the glycocalyx, the passage of larger molecules and interactions between circulating cells and the endothelium are restricted by an intact glycocalyx.^{71,72} Groups are currently working to investigate the function and structure of the cerebral glycocalyx.^{72,73} *In vivo* imaging in mice suggests that the cerebral arteries and capillaries have an intact glycocalyx, whilst veins and venules do not.⁷² As yet we do not know if the same distribution will be seen in the human cerebral circulation. During sepsis patients commonly develop cognitive impairment (acute delirium). The pathogenesis of this condition is complex with evidence to date suggesting a role for cytokines (IL-1, IL-6 and $\text{TNF}\alpha$) penetrating the blood brain barrier and activating astrocytes.⁷⁴ Work by Hippensteel et al now suggests that during sepsis HS fragments penetrate the hippocampal blood brain barrier to inhibit long-term potentiation – the process responsible for memory formation.^{32,75} The blood brain barrier of the hippocampal region appears to be susceptible to damage with an apparent predisposition to age related vascular dysfunction.⁷⁶ It seems possible therefore that an increase in the permeability of the blood brain barrier of the hippocampus to HS fragments, possibly as a direct result of the sepsis-induced glycocalyx shedding, contributed to the hippocampal HS fragment accumulation.³² Interestingly a limited clinical study using SDF imaging has shown that the glycocalyx

depth varies between cortical and hippocampal micro-vessels.⁷³ The hippocampal endothelial glycocalyx was found to be thicker than the cortical endothelial glycocalyx, suggesting that structural differences in the glycocalyx at the two sites may exist.⁷³ Further work is needed to determine whether the hippocampal microvascular glycocalyx is predisposed to damage during sepsis as this could represent an exciting therapeutic target in the future to limit the comorbidity associated with sepsis.

Pre-eclampsia

Pre-eclampsia (PE) is a complication affecting 3-5% of pregnancies. The hallmark of the condition is endothelial cell damage; leading to altered microvascular permeability.^{77,78} Endothelial glycocalyx dysfunction may contribute to this. Activation and dysfunction of the maternal endothelium in PE is mediated (in part) through the release of placentally derived factors.⁷⁹ Hypoxic trophoblasts release anti-angiogenic cytokines including soluble fms-like tyrosine kinase-1 (sFlt-1) into the maternal circulation. In addition there is a reduction in the pro-angiogenic placental growth factor (PlGF) – a member of the VEGF family.⁸⁰ The resulting imbalance contributes to the altered vascular permeability seen in PE.⁸¹ Historically we have shown that VEGF-A, VEGF-C and VEGF-A_{165b} alter the endothelial glycocalyx.^{15,41} Interestingly a failure in first trimester up-regulation of VEGF-A_{165b} has been shown to be predictive for the development of PE.⁸² The important link between VEGF, permeability and the endothelial glycocalyx is an increasing focus in PE research.

The net effect of PE is glycocalyx degradation illustrated by an increased perfused boundary region in sublingual capillaries assessed by side stream imaging in patients with early onset PE.⁸³ However the mechanism of damage remains unclear due to conflicting data to date. HS and HA are both elevated in early-onset PE (onset before 34 weeks'), severe PE and the related syndrome of haemolysis, elevated liver enzymes and low-platelets (HELLP).⁸³⁻⁸⁵ However whilst soluble syndecan-1 has been shown to increase with advancing gestation in both normal pregnancy and in PE, its utility as a marker of PE remains unclear.^{83,86,87} The sample size, diagnostic criteria and different PE sub-types may explain the discrepancies observed. We have recently developed electron microscopy based methodologies to reliably measure the glycocalyx depth in both the foetal and maternal circulation of human placental tissue to aid future work in this area.⁸⁸

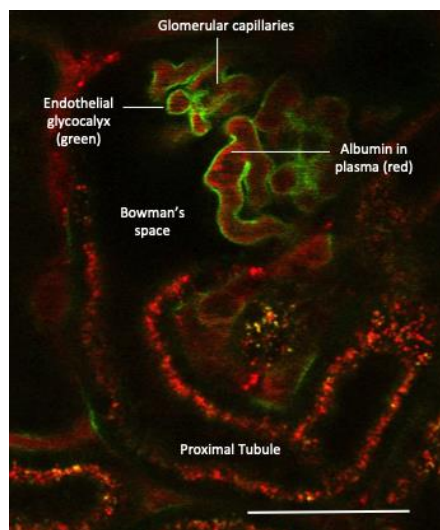
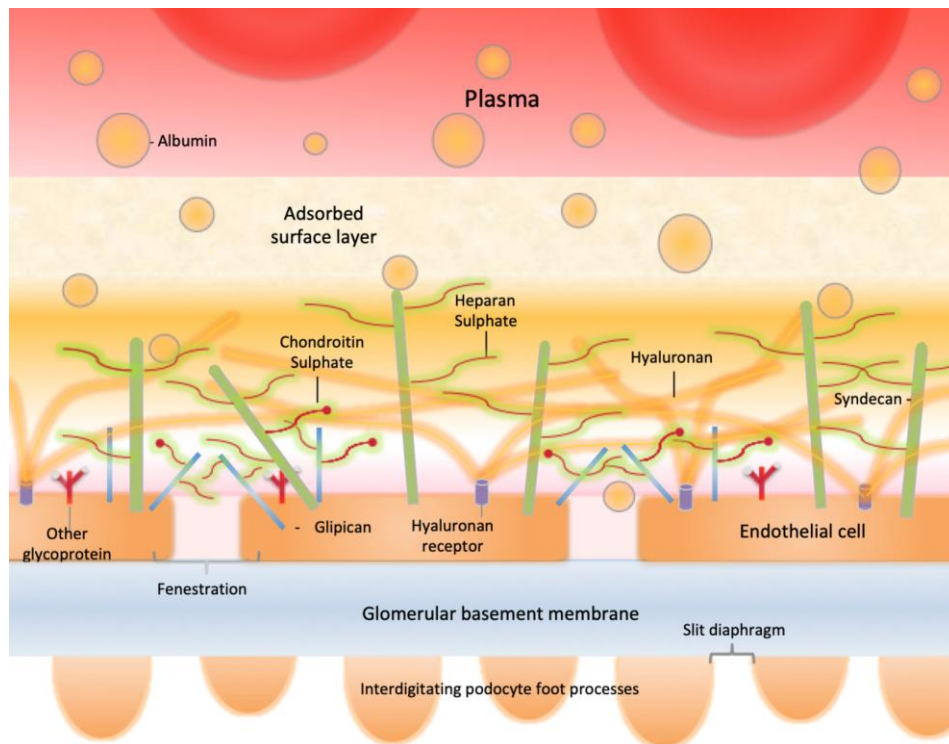
Atherosclerosis

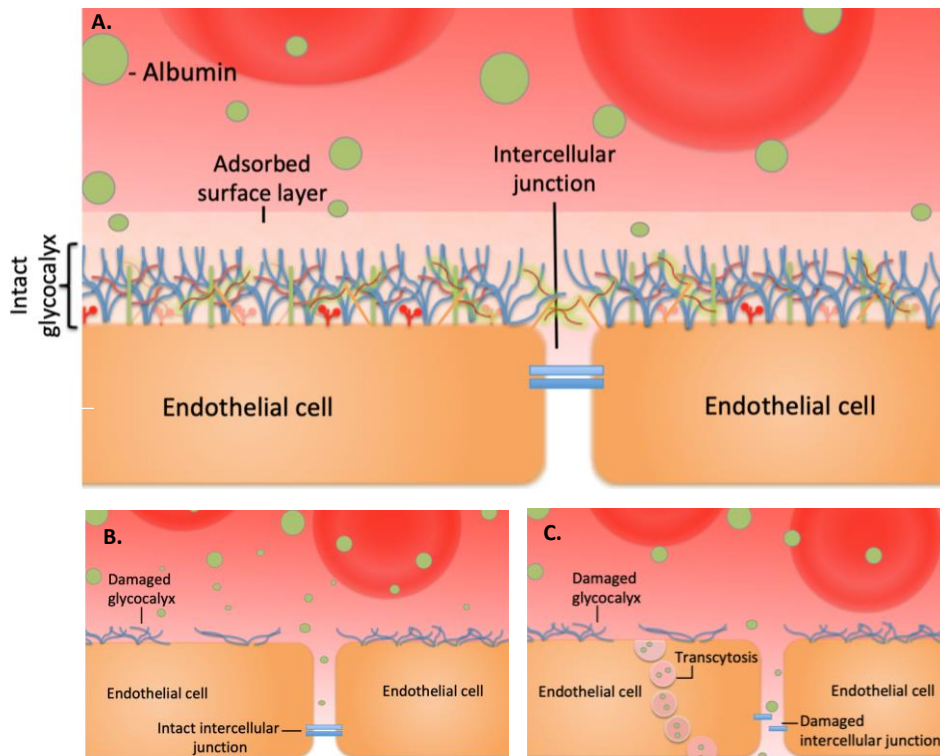
The major focus of this review has been on the function of the endothelial glycocalyx within the microvasculature. However, there is now an emerging field of research studying the role of the glycocalyx in the prevention of atherosclerosis in larger blood vessels. Atherosclerosis is a chronic arterial vascular disease

resulting from lipid-filled plaque accumulation.⁸⁹ The subsequent erosion or rupture of plaques can result in acute arterial occlusion, myocardial infarction or cerebrovascular accident. Damage to the endothelial glycocalyx has been linked to the pathogenesis of atheroma through multiple pathways, which have recently been reviewed by Mitra et al, but alterations in trans-endothelial permeability appear to be a key step.⁸⁹ Atheroma tends to form in areas of non-laminar blood flow adjacent to bends or branch points.^{89,90} *In vitro*, non-uniform fluid flow rapidly results in reduced glycocalyx expression of HS and sialic acids, with an associated reduction in glycocalyx thickness and coverage.⁸⁹ Areas of glycocalyx damage correlated with oxidised low-density lipoprotein (LDL) cholesterol uptake.⁸⁹ *In vivo*, lipid accumulation in apolipoprotein E-deficient (ApoE -/-) mice has been shown to increase in areas of endothelium with incomplete glycocalyx cover (71% glycocalyx cover in regions of plaque vs. 97% cover in plaque free regions)⁹¹ LDL cholesterol uptake is considered important because it triggers cluster of differentiation 40 (CD40/CD40 ligand (L)) signalling pathways and subsequent macrophage uptake and degradation of oxidised LDL results in their transformation into foam cells, a key finding on histological examination of plaques.⁹²

Conclusions

Further research into the structural variability of the glycocalyx and the contribution of the endothelial glycocalyx to vessel wall permeability is needed. This research needs to be targeted to both the organ and vessel of interest. The glycocalyx is a highly specialised structure and pathological states should not be expected to affect remote vascular beds, or the different vessels within them in the same way. Highly sensitive tools like the *ex vivo* glomerular permeability assay are providing new evidence that glycocalyx injury in early disease models has functional consequences and should not be ignored. Glycocalyx damage is associated with increased vessel wall permeability in multiple clinical conditions and has massive potential medical relevance. Restoring or maintaining the glycocalyx represents an inviting therapeutic strategy, but for the full potential of this strategy to be realised we need to gain an increased understanding of how the glycocalyx structure responds to disease and the functional consequences of glycocalyx damage.





Legends

Figure 1. The glomerular filtration barrier The GFB consists of glomerular endothelial cells (GEnC), the glomerular basement membrane and podocytes.^{93,94} GEnC possess numerous transcellular fenestrations, which permit the high hydraulic permeability necessary for filtration, and a glycocalyx which covers the luminal surface, extending over the fenestrations. Podocytes form a second cellular layer by interdigitating their foot processes, which connect at the slit diaphragms. The glycocalyx is a complex structure containing core proteoglycans such as syndecans and glipicans holding glycosaminoglycans (GAGs) heparan sulphate (HS), chondroitin sulphate (CS) and hyaluronan (HA) to the cell surface. The glycocalyx contributes to the filtration barrier by depleting the filtrate protein concentration before it reaches the fenestrated glomerular endothelial cell surface. The generation of a zone of protein-depleted filtrate adjacent to the luminal membrane of endothelial cells (shown in pink) limits loss of macromolecules from the plasma and reduces the effective oncotic pressure across the endothelial cell body.

Figure 2. A live perfused mouse glomerulus imaged under anaesthesia using multiphoton microscopy. Image taken 10 minutes after an intravenous bolus of FITC-wheat germ agglutinin (WGA). This lectin binds to sialic acid residues within the glycocalyx (labelled green). The plasma within the capillary loops was labelled red with AlexaFlour-conjugated albumin. The dark areas within the capillary loops represent circulating blood cells. The glomerular endothelial glycocalyx is a continuous layer within the glomerular capillaries contributing to the restriction of macromolecule and water leakage from the glomerulus into the Bowman's space (Bar = 50µm.) Unpublished image M Butler* Supported NIH grant S10 OD021833 to the USC Multi-Photon Microscopy Core, and MRC grant MR/M018237/1

Figure 3. The double barrier concept

A. In health both the intact glycocalyx and a tight endothelial monolayer can limit vascular wall permeability to macromolecules (including albumin). **B.** When damage to the endothelial barrier is limited to the glycocalyx, in areas of the vasculature where a tight cellular monolayer exists, an intact second barrier remains. This tight endothelial monolayer continues to limit macromolecule transit. In such areas it is currently not possible to directly measure the contribution of the endothelial glycocalyx to the vessel wall permeability. **C.** When damage affects both the glycocalyx and the permeability of the underlying monolayer, marked increases in the vascular wall permeability will result, but calculating the relative contribution of the glycocalyx to the vessel wall permeability, again is not possible. In summary in order to directly measure the contribution of the glycocalyx to vessel permeability vessels lacking a tight endothelial monolayer must be selected until new techniques are developed that can measure macromolecule concentrations in the 'sub-glycocalyx space'.

1. Betteridge, K.B., Arkill, K.P., Neal, C.R., Harper, S.J., Foster, R.R., Satchell, S.C., Bates, D.O. & Salmon, A.H.J. Sialic acids regulate microvessel permeability, revealed by novel in vivo studies of endothelial glycocalyx structure and function. *J Physiol* **595**, 5015-5035 (2017).
2. Butler, M.J., Ramnath, R., Kadoya, H., Desposito, D., Riquier-Brison, A., Ferguson, J.K., Onions, K.L., Ogier, A.S., ElHegni, H., Coward, R.J., Welsh, G.I., Foster, R.R., Peti-Peterdi, J. & Satchell, S.C. Aldosterone induces albuminuria via matrix metalloproteinase-dependent damage of the endothelial glycocalyx. *Kidney Int* **95**, 94-107 (2019).
3. Dane, M.J., van den Berg, B.M., Lee, D.H., Boels, M.G., Tiemeier, G.L., Avramut, M.C., van Zonneveld, A.J., van der Vlag, J., Vink, H. & Rabelink, T.J. A microscopic view on the renal endothelial glycocalyx. *Am J Physiol Renal Physiol* **308**, F956-66 (2015).
4. Singh, A., Satchell, S.C., Neal, C.R., McKenzie, E.A., Tooke, J.E. & Mathieson, P.W. Glomerular endothelial glycocalyx constitutes a barrier to protein permeability. *J Am Soc Nephrol* **18**, 2885-93 (2007).

5. Curry, F.R. Microvascular solute and water transport. *Microcirculation* **12**, 17-31 (2005).
6. Curry, F.E. Layer upon layer: the functional consequences of disrupting the glycocalyx-endothelial barrier in vivo and in vitro. *Cardiovasc Res* **113**, 559-561 (2017).
7. Curry, F.E. & Michel, C.C. The endothelial glycocalyx: Barrier functions versus red cell hemodynamics: A model of steady state ultrafiltration through a bi-layer formed by a porous outer layer and more selective membrane-associated inner layer. *Biorheology* (2019).
8. Potter, D.R. & Damiano, E.R. The hydrodynamically relevant endothelial cell glycocalyx observed in vivo is absent in vitro. *Circ Res* **102**, 770-6 (2008).
9. Chappell, D., Jacob, M., Paul, O., Rehm, M., Welsch, U., Stoeckelhuber, M., Conzen, P. & Becker, B.F. The glycocalyx of the human umbilical vein endothelial cell: an impressive structure ex vivo but not in culture. *Circ Res* **104**, 1313-7 (2009).
10. Edwards, A., Daniels, B.S. & Deen, W.M. Ultrastructural model for size selectivity in glomerular filtration. *Am J Physiol* **276**, F892-902 (1999).
11. Punyaratabandhu, N., Kongoup, P., Dechadilok, P., Katavetin, P. & Triampo, W. Transport of Spherical Particles Through Fibrous Media and a Row of Parallel Cylinders: Applications to Glomerular Filtration. *J Biomech Eng* **139**(2017).
12. Satchell, S. The role of the glomerular endothelium in albumin handling. *Nat Rev Nephrol* **9**, 717-25 (2013).
13. Weinbaum, S., Tarbell, J.M. & Damiano, E.R. The structure and function of the endothelial glycocalyx layer. *Annu Rev Biomed Eng* **9**, 121-67 (2007).
14. Salmon, A.H. & Satchell, S.C. Endothelial glycocalyx dysfunction in disease: albuminuria and increased microvascular permeability. *J Pathol* **226**, 562-74 (2012).
15. Oltean, S., Qiu, Y., Ferguson, J.K., Stevens, M., Neal, C., Russell, A., Kaura, A., Arkill, K.P., Harris, K., Symonds, C., Lacey, K., Wijeyaratne, L., Gammons, M., Wylie, E., Hulse, R.P., Alsop, C., Cope, G., Damodaran, G., Betteridge, K.B., Ramnath, R., Satchell, S.C., Foster, R.R., Ballmer-Hofer, K., Donaldson, L.F., Barratt, J., Baelde, H.J., Harper, S.J., Bates, D.O. & Salmon, A.H. Vascular Endothelial Growth Factor-A165b Is Protective and Restores Endothelial Glycocalyx in Diabetic Nephropathy. *J Am Soc Nephrol* **26**, 1889-904 (2015).
16. Eremina, V., Jefferson, J.A., Kowalewska, J., Hochster, H., Haas, M., Weisstuch, J., Richardson, C., Kopp, J.B., Kabir, M.G., Backx, P.H., Gerber, H.P., Ferrara, N., Barisoni, L., Alpers, C.E. & Quaggin, S.E. VEGF inhibition and renal thrombotic microangiopathy. *N Engl J Med* **358**, 1129-36 (2008).
17. Dimke, H., Maezawa, Y. & Quaggin, S.E. Crosstalk in glomerular injury and repair. *Curr Opin Nephrol Hypertens* **24**, 231-8 (2015).
18. Dane, M.J., van den Berg, B.M., Avramut, M.C., Faas, F.G., van der Vlag, J., Rops, A.L., Ravelli, R.B., Koster, B.J., van Zonneveld, A.J., Vink, H. & Rabelink, T.J. Glomerular endothelial surface layer acts as a barrier against albumin filtration. *Am J Pathol* **182**, 1532-40 (2013).
19. Norden, A.G., Scheinman, S.J., Deschodt-Lanckman, M.M., Lapsley, M., Nortier, J.L., Thakker, R.V., Unwin, R.J. & Wrong, O. Tubular proteinuria defined by a study of Dent's (CLCN5 mutation) and other tubular diseases. *Kidney Int* **57**, 240-9 (2000).
20. Gambaro, G., Baggio, B., Cicerello, E., Mastrosimone, S., Marzaro, G., Borsatti, A. & Crepaldi, G. Abnormal erythrocyte charge in diabetes mellitus. Link with microalbuminuria. *Diabetes* **37**, 745-8 (1988).
21. Ando, Y., Okada, H., Takemura, G., Suzuki, K., Takada, C., Tomita, H., Zaikokuji, R., Hotta, Y., Miyazaki, N., Yano, H., Muraki, I., Kuroda, A., Fukuda, H., Kawasaki, Y., Okamoto, H., Kawaguchi, T., Watanabe, T., Doi, T., Yoshida, T., Ushikoshi, H., Yoshida, S. & Ogura, S.

- Brain-Specific Ultrastructure of Capillary Endothelial Glycocalyx and Its Possible Contribution for Blood Brain Barrier. *Sci Rep* **8**, 17523 (2018).
22. Rehm, M., Zahler, S., Lotsch, M., Welsch, U., Conzen, P., Jacob, M. & Becker, B.F. Endothelial glycocalyx as an additional barrier determining extravasation of 6% hydroxyethyl starch or 5% albumin solutions in the coronary vascular bed. *Anesthesiology* **100**, 1211-23 (2004).
 23. Rops, A.L., Gotte, M., Baselmans, M.H., van den Hoven, M.J., Steenberg, E.J., Lensen, J.F., Wijnhoven, T.J., Cevikbas, F., van den Heuvel, L.P., van Kuppevelt, T.H., Berden, J.H. & van der Vlag, J. Syndecan-1 deficiency aggravates anti-glomerular basement membrane nephritis. *Kidney Int* **72**, 1204-15 (2007).
 24. Garsen, M., Rops, A.L., Rabelink, T.J., Berden, J.H. & van der Vlag, J. The role of heparanase and the endothelial glycocalyx in the development of proteinuria. *Nephrol Dial Transplant* **29**, 49-55 (2014).
 25. Earnshaw, J.S., Curtis, C.G., Powell, G.M., Dodgson, K.S., Olavesen, A.H. & Gacesa, P. The fate of intravenously administered highly purified bovine testicular hyaluronidase (Hyalosidase) in the rat. *Biochem Pharmacol* **34**, 2199-203 (1985).
 26. Landsverk, S.A., Tsai, A.G., Cabrales, P. & Intaglietta, M. Impact of enzymatic degradation of the endothelial glycocalyx on vascular permeability in an awake hamster model. *Crit Care Res Pract* **2012**, 842545 (2012).
 27. Jeansson, M. & Haraldsson, B. Glomerular size and charge selectivity in the mouse after exposure to glucosaminoglycan-degrading enzymes. *J Am Soc Nephrol* **14**, 1756-65 (2003).
 28. Onions, K.L., Gamez, M., Buckner, N.R., Baker, S.L., Betteridge, K.B., Desideri, S., Dallyn, B.P., Ramnath, R.D., Neal, C.R., Farmer, L.K., Mathieson, P.W., Gnudi, L., Alitalo, K., Bates, D.O., Salmon, A.H.J., Welsh, G.I., Satchell, S.C. & Foster, R.R. VEGFC Reduces Glomerular Albumin Permeability and Protects Against Alterations in VEGF Receptor Expression in Diabetic Nephropathy. *Diabetes* **68**, 172-187 (2019).
 29. Gil, N., Goldberg, R., Neuman, T., Garsen, M., Zcharia, E., Rubinstein, A.M., van Kuppevelt, T., Meirovitz, A., Pisano, C., Li, J.P., van der Vlag, J., Vlodavsky, I. & Elkin, M. Heparanase is essential for the development of diabetic nephropathy in mice. *Diabetes* **61**, 208-16 (2012).
 30. Jung, O., Trapp-Stamborski, V., Purushothaman, A., Jin, H., Wang, H., Sanderson, R.D. & Rapraeger, A.C. Heparanase-induced shedding of syndecan-1/CD138 in myeloma and endothelial cells activates VEGFR2 and an invasive phenotype: prevention by novel synstatins. *Oncogenesis* **5**, e202 (2016).
 31. Kim, E.Y., Roshanravan, H. & Dryer, S.E. Syndecan-4 ectodomain evokes mobilization of podocyte TRPC6 channels and their associated pathways: An essential role for integrin signaling. *Biochim Biophys Acta* **1853**, 2610-20 (2015).
 32. Zhang, X., Han, X., Xia, K., Xu, Y., Yang, Y., Oshima, K., Haeger, S.M., Perez, M.J., McMurtry, S.A., Hippensteel, J.A., Ford, J.A., Herson, P.S., Liu, J., Schmidt, E.P. & Linhardt, R.J. Circulating heparin oligosaccharides rapidly target the hippocampus in sepsis, potentially impacting cognitive functions. *Proc Natl Acad Sci U S A* (2019).
 33. Desideri, S., Onions, K.L., Qiu, Y., Ramnath, R.D., Butler, M.J., Neal, C.R., King, M.L.R., Salmon, A.E., Saleem, M.A., Welsh, G.I., Michel, C.C., Satchell, S.C., Salmon, A.H.J. & Foster, R.R. A novel assay provides sensitive measurement of physiologically relevant changes in albumin permeability in isolated human and rodent glomeruli. *Kidney Int* **93**, 1086-1097 (2018).
 34. Nieuwdorp, M., van Haeften, T.W., Gouverneur, M.C., Mooij, H.L., van Lieshout, M.H., Levi, M., Meijers, J.C., Holleman, F., Hoekstra, J.B., Vink, H., Kastelein, J.J. & Strokes, E.S. Loss of endothelial glycocalyx during acute hyperglycemia coincides with endothelial dysfunction and coagulation activation in vivo. *Diabetes* **55**, 480-6 (2006).

35. Lambadiari, V., Pavlidis, G., Kousathana, F., Maratou, E., Georgiou, D., Andreadou, I., Kountouri, A., Varoudi, M., Balampanis, K., Parissis, J., Triantafyllidi, H., Katogiannis, K., Birba, D., Lekakis, J., Dimitriadis, G. & Ikonomidis, I. Effects of Different Antidiabetic Medications on Endothelial Glycocalyx, Myocardial Function, and Vascular Function in Type 2 Diabetic Patients: One Year Follow-Up Study. *J Clin Med* **8**(2019).
36. Targosz-Korecka, M., Jaglarz, M., Malek-Zietek, K.E., Gregorius, A., Zakrzewska, A., Sitek, B., Rajfur, Z., Chlopicki, S. & Szymonski, M. AFM-based detection of glycocalyx degradation and endothelial stiffening in the db/db mouse model of diabetes. *Sci Rep* **7**, 15951 (2017).
37. Satchell, S.C. & Tooke, J.E. What is the mechanism of microalbuminuria in diabetes: a role for the glomerular endothelium? *Diabetologia* **51**, 714-25 (2008).
38. Toyoda, M., Najafian, B., Kim, Y., Caramori, M.L. & Mauer, M. Podocyte detachment and reduced glomerular capillary endothelial fenestration in human type 1 diabetic nephropathy. *Diabetes* **56**, 2155-60 (2007).
39. Tschulakow, A., Christner, S., Julien, S., Ludinsky, M., van der Giet, M. & Schraermeyer, U. Effects of a single intravitreal injection of aflibercept and ranibizumab on glomeruli of monkeys. *PLoS One* **9**, e113701 (2014).
40. Eleftheriadis, T., Antoniadis, G., Pissas, G., Liakopoulos, V. & Stefanidis, I. The renal endothelium in diabetic nephropathy. *Ren Fail* **35**, 592-9 (2013).
41. Foster, R.R., Armstrong, L., Baker, S., Wong, D.W., Wylie, E.C., Ramnath, R., Jenkins, R., Singh, A., Steadman, R., Welsh, G.I., Mathieson, P.W. & Satchell, S.C. Glycosaminoglycan regulation by VEGFA and VEGFC of the glomerular microvascular endothelial cell glycocalyx in vitro. *Am J Pathol* **183**, 604-16 (2013).
42. Singh, A., Friden, V., Dasgupta, I., Foster, R.R., Welsh, G.I., Tooke, J.E., Haraldsson, B., Mathieson, P.W. & Satchell, S.C. High glucose causes dysfunction of the human glomerular endothelial glycocalyx. *Am J Physiol Renal Physiol* **300**, F40-8 (2011).
43. Jeansson, M., Granqvist, A.B., Nystrom, J.S. & Haraldsson, B. Functional and molecular alterations of the glomerular barrier in long-term diabetes in mice. *Diabetologia* **49**, 2200-9 (2006).
44. Satoh, M., Kobayashi, S., Kuwabara, A., Tomita, N., Sasaki, T. & Kashihara, N. In vivo visualization of glomerular microcirculation and hyperfiltration in streptozotocin-induced diabetic rats. *Microcirculation* **17**, 103-12 (2010).
45. Kuwabara, A., Satoh, M., Tomita, N., Sasaki, T. & Kashihara, N. Deterioration of glomerular endothelial surface layer induced by oxidative stress is implicated in altered permeability of macromolecules in Zucker fatty rats. *Diabetologia* **53**, 2056-65 (2010).
46. Kowluru, R.A. & Mishra, M. Regulation of Matrix Metalloproteinase in the Pathogenesis of Diabetic Retinopathy. *Prog Mol Biol Transl Sci* **148**, 67-85 (2017).
47. Wong, T.Y., Cheung, C.M., Larsen, M., Sharma, S. & Simo, R. Diabetic retinopathy. *Nat Rev Dis Primers* **2**, 16012 (2016).
48. Leskova, W., Pickett, H., Eshaq, R.S., Shrestha, B., Pattillo, C.B. & Harris, N.R. Effect of diabetes and hyaluronidase on the retinal endothelial glycocalyx in mice. *Exp Eye Res* **179**, 125-131 (2019).
49. Kumase, F., Morizane, Y., Mohri, S., Takasu, I., Ohtsuka, A. & Ohtsuki, H. Glycocalyx degradation in retinal and choroidal capillary endothelium in rats with diabetes and hypertension. *Acta Med Okayama* **64**, 277-83 (2010).
50. Broekhuizen, L.N., Lemkes, B.A., Mooij, H.L., Meuwese, M.C., Verberne, H., Holleman, F., Schlingemann, R.O., Nieuwdorp, M., Strokes, E.S. & Vink, H. Effect of sulodexide on endothelial glycocalyx and vascular permeability in patients with type 2 diabetes mellitus. *Diabetologia* **53**, 2646-55 (2010).

51. Wallow, I.H. & Engerman, R.L. Permeability and patency of retinal blood vessels in experimental diabetes. *Invest Ophthalmol Vis Sci* **16**, 447-61 (1977).
52. Uchimido, R., Schmidt, E.P. & Shapiro, N.I. The glycocalyx: a novel diagnostic and therapeutic target in sepsis. *Crit Care* **23**, 16 (2019).
53. Chelazzi, C., Villa, G., Mancinelli, P., De Gaudio, A.R. & Adembri, C. Glycocalyx and sepsis-induced alterations in vascular permeability. *Crit Care* **19**, 26 (2015).
54. Fink, M.P. Animal models of sepsis. *Virulence* **5**, 143-53 (2014).
55. Wiesinger, A., Peters, W., Chappell, D., Kentrup, D., Reuter, S., Pavenstadt, H., Oberleithner, H. & Kumpers, P. Nanomechanics of the endothelial glycocalyx in experimental sepsis. *PLoS One* **8**, e80905 (2013).
56. Ramnath, R., Foster, R.R., Qiu, Y., Cope, G., Butler, M.J., Salmon, A.H., Mathieson, P.W., Coward, R.J., Welsh, G.I. & Satchell, S.C. Matrix metalloproteinase 9-mediated shedding of syndecan 4 in response to tumor necrosis factor alpha: a contributor to endothelial cell glycocalyx dysfunction. *FASEB J* (2014).
57. Nieuwdorp, M., Meuwese, M.C., Mooij, H.L., van Lieshout, M.H., Hayden, A., Levi, M., Meijers, J.C., Ince, C., Kastelein, J.J., Vink, H. & Stroes, E.S. Tumor necrosis factor-alpha inhibition protects against endotoxin-induced endothelial glycocalyx perturbation. *Atherosclerosis* **202**, 296-303 (2009).
58. Stepan, J., Hofer, S., Funke, B., Brenner, T., Henrich, M., Martin, E., Weitz, J., Hofmann, U. & Weigand, M.A. Sepsis and major abdominal surgery lead to flaking of the endothelial glycocalyx. *J Surg Res* **165**, 136-41 (2011).
59. Becker, B.F., Jacob, M., Leipert, S., Salmon, A.H. & Chappell, D. Degradation of the endothelial glycocalyx in clinical settings: searching for the sheddases. *Br J Clin Pharmacol* **80**, 389-402 (2015).
60. Yang, X., Meegan, J.E., Jannaway, M., Coleman, D.C. & Yuan, S.Y. A disintegrin and metalloproteinase 15-mediated glycocalyx shedding contributes to vascular leakage during inflammation. *Cardiovasc Res* **114**, 1752-1763 (2018).
61. Lygizos, M.I., Yang, Y., Altmann, C.J., Okamura, K., Hernando, A.A., Perez, M.J., Smith, L.P., Koyanagi, D.E., Gandjeva, A., Bhargava, R., Tudor, R.M., Faubel, S. & Schmidt, E.P. Heparanase mediates renal dysfunction during early sepsis in mice. *Physiol Rep* **1**, e00153 (2013).
62. Cui, N., Wang, H., Long, Y., Su, L. & Liu, D. Dexamethasone Suppressed LPS-Induced Matrix Metalloproteinase and Its Effect on Endothelial Glycocalyx Shedding. *Mediators Inflamm* **2015**, 912726 (2015).
63. Adembri, C., Sgambati, E., Vitali, L., Selmi, V., Margheri, M., Tani, A., Bonaccini, L., Nosi, D., Caldini, A.L., Formigli, L. & De Gaudio, A.R. Sepsis induces albuminuria and alterations in the glomerular filtration barrier: a morphofunctional study in the rat. *Crit Care* **15**, R277 (2011).
64. Koike, K., Aiboshi, J., Shinozawa, Y., Sekine, K., Endo, T. & Yamamoto, Y. Correlation of glomerular permeability, endothelial injury, and postoperative multiple organ dysfunction. *Surg Today* **34**, 811-6 (2004).
65. De Gaudio, A.R., Adembri, C., Grechi, S. & Novelli, G.P. Microalbuminuria as an early index of impairment of glomerular permeability in postoperative septic patients. *Intensive Care Med* **26**, 1364-8 (2000).
66. Matthay, M.A., Zemans, R.L., Zimmerman, G.A., Arabi, Y.M., Beitler, J.R., Mercat, A., Herridge, M., Randolph, A.G. & Calfee, C.S. Acute respiratory distress syndrome. *Nat Rev Dis Primers* **5**, 18 (2019).
67. Schmidt, E.P., Yang, Y., Janssen, W.J., Gandjeva, A., Perez, M.J., Barthel, L., Zemans, R.L., Bowman, J.C., Koyanagi, D.E., Yunt, Z.X., Smith, L.P., Cheng, S.S., Overdier, K.H., Thompson, K.R., Geraci, M.W., Douglas, I.S., Pearse, D.B. & Tudor, R.M. The pulmonary

- endothelial glycocalyx regulates neutrophil adhesion and lung injury during experimental sepsis. *Nat Med* **18**, 1217-23 (2012).
68. Dull, R.O., Mecham, I. & McJames, S. Heparan sulfates mediate pressure-induced increase in lung endothelial hydraulic conductivity via nitric oxide/reactive oxygen species. *Am J Physiol Lung Cell Mol Physiol* **292**, L1452-8 (2007).
 69. Dull, R.O., Cluff, M., Kingston, J., Hill, D., Chen, H., Hoehne, S., Malleske, D.T. & Kaur, R. Lung heparan sulfates modulate K(fc) during increased vascular pressure: evidence for glycocalyx-mediated mechanotransduction. *Am J Physiol Lung Cell Mol Physiol* **302**, L816-28 (2012).
 70. Yang, Y. & Schmidt, E.P. The endothelial glycocalyx: an important regulator of the pulmonary vascular barrier. *Tissue Barriers* **1**(2013).
 71. Galea, I. & Perry, V.H. The blood-brain interface: a culture change. *Brain Behav Immun* **68**, 11-16 (2018).
 72. Yoon, J.H., Lee, E.S. & Jeong, Y. In vivo Imaging of the Cerebral Endothelial Glycocalyx in Mice. *J Vasc Res* **54**, 59-67 (2017).
 73. Haeren, R.H.L., Rijkers, K., Schijns, O., Dings, J., Hoogland, G., van Zandvoort, M., Vink, H. & van Overbeeke, J.J. In vivo assessment of the human cerebral microcirculation and its glycocalyx: A technical report. *J Neurosci Methods* **303**, 114-125 (2018).
 74. Piva, S., McCreadie, V.A. & Latronico, N. Neuroinflammation in sepsis: sepsis associated delirium. *Cardiovasc Hematol Disord Drug Targets* **15**, 10-8 (2015).
 75. Hippensteel, J.A., Anderson, B.J., Orfila, J.E., McMurtry, S.A., Dietz, R.M., Su, G., Ford, J.A., Oshima, K., Yang, Y., Zhang, F., Han, X., Yu, Y., Liu, J., Linhardt, R.J., Meyer, N.J., Herson, P.S. & Schmidt, E.P. Circulating heparan sulfate fragments mediate septic cognitive dysfunction. *J Clin Invest* **129**, 1779-1784 (2019).
 76. Montagne, A., Barnes, S.R., Sweeney, M.D., Halliday, M.R., Sagare, A.P., Zhao, Z., Toga, A.W., Jacobs, R.E., Liu, C.Y., Amezcua, L., Harrington, M.G., Chui, H.C., Law, M. & Zlokovic, B.V. Blood-brain barrier breakdown in the aging human hippocampus. *Neuron* **85**, 296-302 (2015).
 77. Boeldt, D.S. & Bird, I.M. Vascular adaptation in pregnancy and endothelial dysfunction in preeclampsia. *J Endocrinol* **232**, R27-r44 (2017).
 78. Tranquilli, A.L., Dekker, G., Magee, L., Roberts, J., Sibai, B.M., Steyn, W., Zeeman, G.G. & Brown, M.A. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. *Pregnancy Hypertens* **4**, 97-104 (2014).
 79. O'Brien, M., Baczyk, D. & Kingdom, J.C. Endothelial Dysfunction in Severe Preeclampsia is Mediated by Soluble Factors, Rather than Extracellular Vesicles. *Sci Rep* **7**, 5887 (2017).
 80. Levine, R.J., Maynard, S.E., Qian, C., Lim, K.H., England, L.J., Yu, K.F., Schisterman, E.F., Thadhani, R., Sachs, B.P., Epstein, F.H., Sibai, B.M., Sukhatme, V.P. & Karumanchi, S.A. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* **350**, 672-83 (2004).
 81. Maynard, S.E., Min, J.-Y., Merchan, J., Lim, K.-H., Li, J., Mondal, S., Libermann, T.A., Morgan, J.P., Sellke, F.W., Stillman, I.E., Epstein, F.H., Sukhatme, V.P. & Karumanchi, S.A. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *Journal of Clinical Investigation* **111**, 649-658 (2003).
 82. Bills, Victoria L., Varet, J., Millar, A., Harper, Steven J., Soothill, Peter W. & Bates, David O. Failure to up-regulate VEGF(165)b in maternal plasma is a first trimester predictive marker for pre-eclampsia. *Clinical Science (London, England : 1979)* **116**, 265-272 (2009).

83. Weissgerber, T.L., Garcia-Valencia, O., Milic, N.M., Codsí, E., Cubro, H., Nath, M.C., White, W.M., Nath, K.A. & Garovic, V.D. Early Onset Preeclampsia Is Associated With Glycocalyx Degradation and Reduced Microvascular Perfusion. *J Am Heart Assoc* **8**, e010647 (2019).
84. Osmers, R.G., Schutz, E., Diedrich, F., Wehry, B., Krauss, T., Oellerich, M. & Kuhn, W. Increased serum levels of hyaluronic acid in pregnancies complicated by preeclampsia or hemolysis, elevated liver enzymes, and low platelets syndrome. *Am J Obstet Gynecol* **178**, 341-5 (1998).
85. Berg, S., Engman, A., Holmgren, S., Lundahl, T. & Laurent, T.C. Increased plasma hyaluronan in severe pre-eclampsia and eclampsia. *Scand J Clin Lab Invest* **61**, 131-7 (2001).
86. Hofmann-Kiefer, K.F., Knabl, J., Martinoff, N., Schiessl, B., Conzen, P., Rehm, M., Becker, B.F. & Chappell, D. Increased serum concentrations of circulating glycocalyx components in HELLP syndrome compared to healthy pregnancy: an observational study. *Reprod Sci* **20**, 318-25 (2013).
87. Gandley, R.E., Althouse, A., Jeyabalan, A., Bregand-White, J.M., McGonigal, S., Myerski, A.C., Gallaher, M., Powers, R.W. & Hubel, C.A. Low Soluble Syndecan-1 Precedes Preeclampsia. *PLoS One* **11**, e0157608 (2016).
88. Fabre-Gray, A.C.M., Down, C.J., Neal, C.R., Foster, R.R., Satchell, S.C. & Bills, V.L. Imaging the placental glycocalyx with transmission electron microscopy. *Placenta* **74**, 59-61 (2018).
89. Mitra, R., O'Neil, G.L., Harding, I.C., Cheng, M.J., Mensah, S.A. & Ebong, E.E. Glycocalyx in Atherosclerosis-Relevant Endothelium Function and as a Therapeutic Target. *Curr Atheroscler Rep* **19**, 63 (2017).
90. Zand, T., Majno, G., Nunnari, J.J., Hoffman, A.H., Savelonis, B.J., MacWilliams, B. & Joris, I. Lipid deposition and intimal stress and strain. A study in rats with aortic stenosis. *Am J Pathol* **139**, 101-13 (1991).
91. Cancel, L.M., Ebong, E.E., Mensah, S., Hirschberg, C. & Tarbell, J.M. Endothelial glycocalyx, apoptosis and inflammation in an atherosclerotic mouse model. *Atherosclerosis* **252**, 136-146 (2016).
92. Parthasarathy, S., Raghavamenon, A., Garelnabi, M.O. & Santanam, N. Oxidized low-density lipoprotein. *Methods Mol Biol* **610**, 403-17 (2010).
93. Satchell, S.C., Tasman, C.H., Singh, A., Ni, L., Geelen, J., von Ruhland, C.J., O'Hare, M.J., Saleem, M.A., van den Heuvel, L.P. & Mathieson, P.W. Conditionally immortalized human glomerular endothelial cells expressing fenestrations in response to VEGF. *Kidney Int* **69**, 1633-40 (2006).
94. Deen, W.M., Lazzara, M.J. & Myers, B.D. Structural determinants of glomerular permeability. *Am J Physiol Renal Physiol* **281**, F579-96 (2001).